Bacteriological Analytical Manual *Online*

January 2001

M154

Trypticase (Tryptic) Soy Broth

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Trypticase peptone	17 g
Phytone peptone	3 g
NaCl	5 g
K_2HPO_4	2.5 g
Glucose	2.5 g
Distilled water	1 liter

Heat with gentle agitation to dissolve. Dispense 225 ml into 500 ml Erlenmeyer flasks. Autoclave 15 min at 121°C. Final pH, 7.3 ± 0.2.

For trypticase soy broth without glucose, prepare as above, but omit 2.5 g glucose.

For use with halophilic *Vibrio* spp., add NaCl to a final concentration of 2-3%.

Hypertext Source: Bacteriological Analytical Manual, 8th Edition, Revision A, 1998.

Expected Results

Suspensions containing large numbers of bacterial spores are obtained with the use of AK Agar #2.

Availability

BBL™ AK Agar #2 (Sporulating Agar)
Cat. No. 210912 Dehydrated – 500 g

References

1. Arret and Kirshbaum. 1959. J. Milk Food Technol. 22:329.
2. Richardson (ed.). 1985. Standard methods for the examination of dairy products, 15th ed. American Public Health Association. Washington, D.C.

APT Agar • APT Broth

Intended Use

APT Agar is used for cultivating heterofermentative lactobacilli and other organisms requiring high thiamine content. It is also used for maintaining stock cultures of *Weissella (Lactobacillus) viridescens* ATCC™ 12706 used in the assay of thiamine.

APT Broth is used for culturing *Weissella viridescens* ATCC 12706 used in the assay of thiamine. It is also used for cultivating heterofermentative lactobacilli and other organisms requiring high thiamine content.

Summary and Explanation

Evans and Niven[1] investigated cultivating the heterofermentative lactobacilli that cause the faded or greenish discoloration of cured meat products, while Deibel, Evans and Niven[2] investigated thiamine requiring bacteria, specifically *Lactobacillus viridescens*. Their formulations led to the development of APT Agar and APT Broth.

Historically, the lactic acid bacteria, a group of acid-producing bacteria, included the genera *Streptococcus*, *Leuconostoc*, *Pediococcus* and *Lactobacillus*; currently, taxonomists include a number of additional genera (e.g., *Weissella*).[3] These organisms are widespread in nature and are associated with bacterial spoilage of foods such as dairy, meat and vegetable products.[3] One use of APT Agar and APT Broth is for cultivating these heterofermentative lactic acid bacteria from food products.[3]

APT Agar and APT Broth are also used in the microbiological assay of thiamine. In the assay, APT Agar is the maintenance medium that preserves the viability and sensitivity of *Weissella viridescens* ATCC 12706. APT Broth is used for growing *Weissella viridescens* ATCC 12706 and preparing the inoculum.

User Quality Control

Identity Specifications

Difco™ APT Agar

Dehydrated Appearance:	Light beige, free-flowing, homogeneous.
Solution:	6.12%, soluble in purified water upon boiling. Solution is medium amber, clear to slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Medium amber, clear to slightly opalescent, may have a slight precipitate.
Reaction of 6.12% Solution at 25°C:	pH 6.7 ± 0.2

Difco™ APT Broth

Dehydrated Appearance:	Light tan, free-flowing, homogeneous.
Solution:	4.62%, soluble in purified water with slight heating. Solution is opalescent when hot. After cooling, is light to medium amber, clear to very slightly opalescent, may have a slight precipitate.
Prepared Appearance:	Light to medium amber, clear to very slightly opalescent without significant precipitate.
Reaction of 4.62% Solution at 25°C:	pH 6.7 ± 0.2

Cultural Response

Difco™ APT Agar or APT Broth

Prepare the medium per label directions. Inoculate and incubate at 35 ± 2°C for 24-48 hours.

ORGANISM	ATCC™	INOCULUM CFU	RECOVERY
Lactobacillus fermentum	9338	10^2-10^3	Good
Weissella viridescens	12706	10^2-10^3	Good

Principles of the Procedure

APT Agar and APT Broth contain peptone as a source of carbon, nitrogen, vitamins and minerals. Yeast extract supplies B-complex vitamins which stimulate bacterial growth. Dextrose is the carbohydrate. The manganese chloride, magnesium sulfate and ferrous sulfate provide ions used in replication by lactobacilli. Polysorbate 80 is a source of fatty acids required by lactobacilli. Agar is the solidifying agent in APT Agar.

Formulae

Difco™ APT Agar

Approximate Formula* Per Liter

Yeast Extract	7.5 g
Pancreatic Digest of Casein	12.5 g
Dextrose	10.0 g
Sodium Citrate	5.0 g
Thiamine Hydrochloride	1.0 mg
Sodium Chloride	5.0 g
Dipotassium Phosphate	5.0 g
Manganese Chloride	0.14 g
Magnesium Sulfate	0.8 g
Ferrous Sulfate	0.04 g
Polysorbate 80	0.2 g
Agar	15.0 g

Difco™ APT Broth

Consists of the same ingredients without the agar.

**Adjusted and/or supplemented as required to meet performance criteria.*

Directions for Preparation from Dehydrated Product

Difco™ APT Agar

1. Suspend 61.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Heat with frequent agitation and boil for 1 minute to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Test samples of the finished product for performance using stable, typical control cultures.

Difco™ APT Broth

1. Suspend 46.2 g of the powder in 1 L of purified water. Mix thoroughly.
2. Warm slightly to completely dissolve the powder.
3. Autoclave at 121°C for 15 minutes. Avoid overheating.
4. Test samples of the finished product for performance using stable, typical control cultures.

Procedure

For maintaining stock cultures of *Weissella viridescens* ATCC 12706 prepare a stab inoculation. Prepare stock cultures in triplicate at monthly intervals. One of the transfers is saved for the preparation of stock cultures. The others are used to prepare inoculum in APT Broth for assay as needed. Following incubation at 35-37°C for 24-48 hours, store stock cultures at 2-8°C.

Expected Results

Refer to appropriate references and procedures for results.

References

1. Evans and Niven. 1951. J. Bacteriol. 62:599.
2. Deibel, Evans and Niven. 1957. J. Bacteriol. 74:818.
3. Hall, Ledenbach and Flowers. 2001. *In* Downes and Ito (ed.), Compendium of methods for the microbiological examination of foods, 4th ed. American Public Health Association, Washington, D.C.

Availability

Difco™ APT Agar

COMPF USDA

Cat. No. 265430 Dehydrated – 500 g

Difco™ APT Broth

Cat. No. 265510 Dehydrated – 500 g

Acetamide Agar

Intended Use

Acetamide Agar is used in the differentiation of nonfermentative gram-negative bacteria, particularly *Pseudomonas aeruginosa*.

Summary and Explanation

Assimilation studies by Gilardi and others using basal mineral media showed that acetamide was utilized by a wide variety of nonfermenting organisms.[1,2] However, few organisms are reported to deaminate acetamide.[3,4] A variety of media formulations have been developed to determine the ability of various nonfermenting gram-negative organisms to deaminate acetamide for purposes of identification.[5-8] The formulation of this medium is the one recommended in *Standard Methods for the Examination of Water and Wastewater.*[9]

Principles of the Procedure

The ability to deaminate acetamide (acylamidase activity) has been found to be most actively accomplished by *P. aeruginosa, Comamonas acidovorans, Achromobacter xylosoxidans* subsp. *xylosoxidans* (*Alcaligenes xylosoxidans*) and *Alcaligenes faecalis (odorans).*[8] Deamination of acetamide produces ammonia which increases the pH of the medium causing a corresponding color change from yellow-orange to purplish-red.

Procedure

Inoculate the Acetamide Agar slant with a loopful of culture emulsified in BBL™ Trypticase™ Soy Broth. Incubate inoculated slant at 35 ± 2°C and observe daily for 4 days and again at 7 days before discarding as negative.

Expected Results

Deamination of the acetamide is indicated by a pronounced purplish-red color of the medium.

Complete identification requires determination of the Gram reaction, cellular morphology, biochemical reactions, etc. Appropriate references may be consulted for further information.[10, 11]

Limitations of the Procedure

Some strains deaminate acetamide slowly and may require as long as 7 days to yield a positive test result.

Only about 37% of apyocyanogenic strains of *P. aeruginosa* will produce a positive reaction. Therefore, this test should not be relied upon as a sole criterion for identification.[11]

References

1. Gilardi. 1974. Antonie van Leewenhoek. J. Microbiol. Serol. 39:229.
2. Stainier, Palleroni and Doudoroff. 1966. J. Gen. Microbiol. 43:159.
3. Pickett and Pedersen. 1970. Can. J. Microbiol. 16:351.
4. Pickett and Pedersen. 1970. Can. J. Microbiol. 16:401.
5. Hedberg. 1969. Appl. Microbiol. 17:481.
6. Smith and Dayton. 1972. Appl. Microbiol. 24:143.
7. Buhlmann, Vischer and Bruhin. 1961. J. Bacteriol. 82:787.
8. Oberhofer and Rowen. 1974. Appl. Microbiol. 28:720.
9. Clesceri, Greenberg and Eaton (ed.) 1998. Standard methods for the examination of water and wastewater, 20th ed. American Public Health Association, Washington, D.C.
10. Holt, Krieg, Sneath, Staley and Williams (ed.). 1994. Bergey's Manual™ of determinative bacteriology, 9th ed. Williams & Wilkins, Baltimore, Md.
11. Murray, Baron, Pfaller, Tenover and Yolken (ed.). 1999. Manual of clinical microbiology, 7th ed. American Society for Microbiology, Washington, D.C.

Availability

BBL™ Acetamide Agar

SMWW

Cat. No. 221828 Prepared Slants – Pkg. of 10*

*Store at 2-8°C.

APT BROTH BASE

INTENDED USE
REMEL's APT Broth Base is a medium recommended for use in qualitative procedures for the cultivation of lactic acid bacteria and other organisms requiring high thiamine content.

SUMMARY AND EXPLANATION
APT Broth is patterned after the formulation of Evans and Niven and Deibel, Evans, and Niven.[1,2] APT Broth may be used for the enumeration and cultivation of lactic acid bacteria. Since the medium is nonselective, coliforms and many common bacteria will grow on it as well. It is used according to the procedures listed in the *Compendium of Methods for the Microbiological Examination of Foods* by the American Public Health Association (APHA).[3]

PRINCIPLE
Casein peptone supplies essential amino acids, minerals, and nitrogenous compounds. Dextrose is the energy source, and yeast extract supplies B-complex vitamins and serves as a growth enhancer. Sodium chloride maintains osmotic equilibrium. Metallic salts are sources of ions essential for the replication of lactic acid bacteria. Polysorbate 80, which is a mixture of oleic esters, is a source of fatty acids required by lactobacilli.

REAGENTS (CLASSICAL FORMULA)*

Casein Peptone	12.5 g	Magnesium Sulfate	0.8 g
Yeast Extract	7.5 g	Manganous Chloride	0.14 g
Sodium Chloride	5.0 g	Ferrous Sulfate	0.04 g
Potassium Phosphate	5.0 g	Dextrose	10.0 g
Sodium Citrate	5.0 g	Thiamine Hydrochloride	0.001 g
Polysorbate 80	0.2 g		

pH 6.7 +/- 0.2 @ 25°C

*Adjusted as required to meet performance standards.

PRECAUTIONS
This product is *For Laboratory Use* only. It is not intended for use in the diagnosis of disease or other conditions.

PREPARATION OF DEHYDRATED CULTURE MEDIUM
1. Suspend 46.2 g of medium in 1000 ml of demineralized water.
2. Warm to dissolve if necessary.
3. Dispense into appropriate containers and sterilize by autoclaving at 121°C for 15 minutes.

PROCEDURE
1. Consult appropriate references for the recommended procedure for sample inoculation and cultivation.
2. Incubate sample aerobically for 18-24 hours at 35-37°C.
3. Observe broth for turbidity.
4. Subculture turbid broth to nonselective and selective media.

QUALITY CONTROL
Each lot number of APT Broth Base has been manufactured, packaged, and processed in accordance with current Good Manufacturing Practices. All lot numbers have been tested using the following quality control organisms and have been found to be acceptable. Testing of control organisms should be performed in accordance with established laboratory quality control procedures.

CONTROL	INCUBATION	RESULTS
Lactobacillus johnsonii ATCC® 33200	Aerobic, up to 48 h @ 35°C	Growth
Lactobacillus rhamnosus ATCC® 9595	Aerobic, up to 48 h @ 35°C	Growth
Lactococcus lactis ATCC® 19257	Aerobic, up to 48 h @ 35°C	Growth
Leuconostoc mesenteroides ATCC® 17071	Aerobic, up to 48 h @ 35°C	Growth

BIBLIOGRAPHY

1. Evans, J.B. and C.F. Niven, Jr. 1951. J. Bacteriol. 62:599-603.
2. Deibel, R.H., J.B. Evans, and C.F. Niven, Jr. 1957. J. Bacteriol. 74:818-821.
3. Downes, F.P. and K. Ito. 2001. Compendium of Methods for the Microbiological Examination of Foods. 4th ed. APHA, Washington, D.C.
4. MacFaddin, J.F. 1985. Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Williams & Wilkins, Baltimore, MD.

Refer to the front of the manual for **General Information** regarding precautions, product storage and deterioration, specimen collection, storage and transportation, materials required, quality control, and limitations.

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remel Apogent

12076 Santa Fe Drive, Lenexa, KS 66215, USA
General Information: (800) 255-6730 Technical Service: (800) 447-3641 Order Entry: (800) 447-3635
Local/International Phone: (913) 888-0939 International Fax: (913) 895-4128
Website: www.remel.com Email: remel@remel.com

22092 Tryptic Soy Broth

Fluka ***BioChemika*, for microbiology**

Synonym
Casein Soya Broth
CASO Broth
Soybean Casein digest Broth
Tryptone Soya Broth
TSB

Expand/Collapse All

Price and Availability

Product Number	Your Price USD	Available to Ship		Quantity	Actions
22092-100G	11.00	04/17/2006	details...		
22092-500G	29.30	date not available	details...		

Descriptions

Application
For confirmation of *Campylobacter jejuni* by means of the motility test.
Recommended by the "Schweizerischen Lebensmittelbuch" 5th ed., chapter 56A.

Other Notes
Capability of selective media to detect heat-injured *Shigella flexneri*[1]

Properties

grade for microbiology

product line *BioChemika*

shelf life (limited shelf life, expiry date on the label)

composition casein peptone (pancreatic), 17 g/L
dipotassium hydrogen phosphate, 2.5 g/L
glucose, 2.5 g/L
sodium chloride, 5 g/L
soya peptone (papain digest.), 3 g/L

final pH 7.3±0.2 (25 °C)

References

Cited References
1. J.L. Smith, B.J. Dell *J. Food Prot.* **53**, 141, (1990)

Safety

WGK Germany 3

F 3-21

Related Categories

... Media > **Broths**

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7. Anon., J. Food Microbiol. 5. 291 (1987)
8. Lee K., Baron E.J., Summanen P. and Finegold S., J. Clin. Microbiol. 28. 1747 (1990)
9. Beumer R.R., te Giffel M.C. and Cox L.J., Lett. Appl. Microbiol. 24. 421 (1997)

22092 Tryptic Soy Broth (TSB, Tryptone Soya Broth, CASO Broth, Soybean Casein digest Broth, Casein Soya Broth)

The medium will support a luxuriant growth of many fastidious organisms without the addition of serum.
Used for confirmation of Campylobacter jejuni by means of the motility test. Recommended by the "Schweizerisches Lebensmittelbuch" 5th ed., chapter 56A, USP XXIII (1995), EP (1999) and the Ph Eur. (1999).

Composition:

Ingredients	Grams/Litre
Casein peptone (pancreatic)	17.0
Soya peptone (papain digest.)	3.0
Sodium chloride	5.0
Dipotassium hydrogen phosphate	2.5
Glucose	2.5
Final pH 7.3 +/- 0.2 at 37°C	

Store prepared media below 8°C, protected from direct light. Store dehydrated powder, in a dry place, in tightly-sealed containers at 2-25°C.

Directions:
Suspend 30 g of dehydrated media in 1 litre of purified filtered water. Sterilize at 121°C for 15 minutes.

Principle and Interpretation:
Casein peptone and Soya peptone provide nitrogen, vitamins and minerals. The natural sugars from Soya peptone and Glucose promote organism growth. Sodium chloride is for the osmotic balance, while Dipotassium hydrogen phosphate is a buffering agent.
Tryptone Soya Broth is often for the tube dilution method of antibiotic susceptibility testing. The addition of a small amount of agar (approx. 0.05-0.2% Fluka 05040, add before sterilisation) renders the broth suitable for the cultivation of obligatory anaerobes, such as Clostridium species. The superior growth-promoting properties of Tryptic Soy Broth make it especially useful for the isolation of organisms from blood or other body fluids. Anticoagulants such as sodium polyanetholesulfonate (Fluka 81305) or sodium citrate (Fluka 71635) may be added to the broth prior to sterilisation. 5 to 10 ml of blood may be added to 50 ml of medium.

Cultural characteristics after 18-48 hours at 35°C (if necessary 76 hours).

Organisms (ATCC)	Growth	max. incubation time in days
Escherichia coli (8739)	+++	3
Staphylococcus aureus (6538-P)	+++	3
Streptococcus pneumoniae (6301)	+++	3
Bacillus subtilis (6633)	+++	3
Pseudomonas aeruginosa (9027)	+++	3
Candida albicans (2091 or 10231)	+++	5
Aspergillus niger (6301)	+++	5

References:

1. J.L. Smith, B.J. Dell, Capability of selective media to detect heat –injured Shigella flexneri, J. Food Protect. 53, 141 (1990)
2. R.G. Garison, Studies of the respiratory activity of Histoplasma Capsulatum, J. of infect.. Dis. 108: 120-124 (1961)
3. N.B. Mc Culloug, Laboratory tests in the diagnosis of brucellosis. Amer. J. of puplic health 39: 866-869 (1949)
4. Jean. F. Mac Faddin, Media for Isolation-Cultivation-Identification-Maintenance of Medical Bacteria. Vol. 1. Baltimore, MD.: Williams & Wilkins. (1985)